Perinatal outcomes after fresh versus vitrified-warmed blastocyst transfer: retrospective analysis

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Objective: To investigate the possible effect of controlled ovarian stimulation on the perinatal outcomes of assisted reproductive technology pregnancies, by comparing the outcomes from fresh ET with frozen ET (FET) with blastocysts of similar quality.
Design: Retrospective observational study.
Setting: Private fertility center.
Patient(s): Seven hundred eighty-four fresh transfers and 382 vitrified-warmed double blastocyst transfers.
Intervention(s): None.
Main Outcome Measure(s): Miscarriage, perinatal mortality, preterm delivery, live birth, live-birth weights, and gestational age of live births.
Result(s): FET resulted in higher implantation rates (51.5% vs. 40.6%), higher live-birth rates per transfer (56.8% vs. 44.3%), and lower ectopic pregnancy rates (0.32% vs. 1.80%). FET pregnancies also had higher day 14 βhCG levels per implantation (148.2 vs. 176.2 IU/L) and higher infant birth weights (singletons Δ109.4 g, twins Δ124 g). Female infants benefitted the most in terms of birth weight. Miscarriage, premature delivery, perinatal morbidity, and live birth per pregnancy were all nonsignificantly different between fresh ET and FET.
Conclusion(s): Clinically significant differences between the peri-implantation and perinatal outcomes of fresh ET and FET suggest better endometrial receptivity and placentation in FET cycles. (Fertil Steril® 2015;104:899–907. ©2015 by American Society for Reproductive Medicine.)
Key Words: COS, fresh ET, HRT-FET, blastocysts, vitrification, pregnancy, perinatal outcomes

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Assisted reproductive technology (ART) using controlled ovarian stimulation (COS) and fresh ETs has until now been hampered by relatively low embryo implantation rates and an increased risk of ovarian hyperstimulation syndrome (OHSS). In addition, a number of epidemiological and population-based studies have recently suggested that COS followed by fresh transfer may result in pregnancies at increased risk of adverse perinatal, neonatal, and long-term health outcomes relative to pregnancies and infants resulting from spontaneous conceptions (1–6).

In the past, these adverse reproductive outcomes (preterm delivery, infant morbidity, and mortality) after ART were mostly attributed to multiple gestations, owing to multiple ETs. A recent study has, however, shown that even singleton ART pregnancies are at increased risk of adverse outcomes (7).

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The increasing use of single ET (SET) with its concomitant reduction in pregnancy rates has encouraged investigations into other strategies to maintain pregnancy outcomes at levels comparable to those of multiple ETs. The cryopreservation of superfluous viable embryos and their transfer in subsequent frozen ET (FET) cycles is one such strategy. The cumulative pregnancy rates from a combined SET strategy have been shown to be similar to those of multiple ETs (4, 8), however, with the added advantage of a reduction in multiple pregnancy rates. The development of more effective cryotechnologies, and in particular vitrification technologies, has resulted in improved embryo survival rates and pregnancy rates from FET (9),

apparently without compromising the health of infants born from the FET technology (10, 11). As a consequence, the most recent studies in which fresh blastocyst transfer was compared with vitrified-warmed blastocysts transfer reported equivalent or even better pregnancy rates from the vitrified-warmed blastocyst transfers (12, 13).

ART pregnancy and obstetric outcomes have been shown to be independently associated with specific patient demographics, that is, age, parity, cause, and duration of infertility as well as confounders related to the ART procedure, that is, the COS protocol and embryo culture methods (6, 7). Of the ART-related confounding factors, COS has received the most attention owing to its ubiquitous use and the well-publicized effects of its resultant supraphysiological luteal steroid levels. The early luteal phase is critical to pregnancy, as it governs embryo implantation and placentation (3, 14). The elucidation of the cause and effect relationship between COS and pregnancy outcomes has, therefore, been the key objective of a number of studies, each with a different approach (7, 15–20).

The possible detrimental effects of COS can, however, be circumvented by not transferring embryos into the luteal conditions encountered after COS but to rather cryopreserve all embryos and transfer them in subsequent natural or hormone therapy (HT) FET cycles. The physiological intrauterine conditions of FET may have a positive influence not only on the endometrial receptivity and early implantation but also on placentation and subsequent fetal growth (4). These assumptions have in general been substantiated by recent perinatal comparative studies showing that births from FET are characterized by better perinatal characteristics (i.e., on average, longer duration of gestation, higher birth weight) and with similar neonatal and birth defect outcomes compared with fresh ET (1, 7). Specifically, the outcomes of preterm birth, very preterm birth, small for gestational age, low birth weight (LBW), and perinatal mortality have all been reported to be lower in FET pregnancies (8, 21–23). Interestingly, the birth weights and preterm birth rates of singleton FET pregnancies have been found to be similar to those of singleton spontaneous conceptions (7).

The use of vitrified-warmed blastocyst transfer to improve implantation rates is of no benefit to patients if the cryopreservation technologies and methodologies used may in some way adversely affect the neonatal and long-term health outcomes of infants born from FET. The present study was therefore performed to investigate which of the two blastocyst-transfer strategies, fresh ET or FET, would provide the best possible outcomes in terms of peri-implantation and perinatal outcomes.

MATERIALS AND METHODS

Study Details

In this retrospective observational analysis we compared the implantation and perinatal outcomes of fresh double blastocyst ET with vitrified-warmed double blastocyst FET. The transfer cycles included in the study were performed at Antalya IVF during the period January 2012 to December 2013. The study was initiated after the implementation of vitrification as the routine method for cryopreservation in June 2011, and as more than 90% of the subsequent vitrified-warmed transfers were double blastocyst transfers, only double blastocyst transfers were included in the study. The study was approved by the Institutional Review Board and ethics committee of the Akdeniz University, Medical Faculty (reference no. 443/2014). The clinical procedures described in the study were performed by the three resident clinicians of Antalya IVF. The patient cycles were extracted without patient identifiers from the ART database of Antalya IVF, with a patient included only once in a group. However, 42 patients were included in the fresh ET group and in the FET group because the patients had a failed fresh cycle and a subsequent FET cycle that met the study inclusion criteria. The inclusion criteria were primarily based on blastocyst outcomes of cycles. To be included in the fresh ET group, cycles had to have at least two day 5 blastocysts, one with an expansion grade of ≥ 3, an inner cell mass (ICM) grade of A or B, and a trophectoderm (TE) grade of A or B. Likewise, to be included in the FET group, cycles had to have at least two survivng day 5 blastocysts, one with an expansion grade of ≥ 3, an ICM grade of A or B, and a TE grade of A or B. On the basis of the aforementioned inclusion criteria, a total of 821 fresh ET and 421 FET cycles were initially selected for comparison.

Ovarian Stimulation and Embryo Culture

All COS were GnRH antagonist (Cetrotide, Merck Serono) cotreatment protocols, with rFSH (Gonal-F, Merck Serono) and hMG (Menopur, Ferring Pharmaceuticals) for follicular stimulation and GnRH agonist (Gonapeptyl, Ferring Pharmaceuticals) or hCG (Ovitrelle, Merck Serono) for ovulation induction. A transvaginal ultrasound–guided oocyte pickup (OPU) procedure was performed 36 hours after ovulation induction. All gamete manipulations and embryo cultures were performed using Sydney IVF (COOK Medical, Sidney IVF) media. Incubation conditions were set at 6% CO_2, 5% O_2, and 37.0°C (K-Systems, Kivex Biotec). All oocyte inseminations were performed using intracytoplasmic sperm injection. Embryos were cultured in microdroplets of media overlaid with light mineral oil. All embryos were cultured to at least the fifth day after OPU for transfer and or cryopreservation. Blastocysts were assessed according to the three-part grading system proposed by Gardner and Schoolcraft (24, 25): blastocyst expansion on a scale of 1 to 6; the ICM on a scale of A to C, according to the number and degree of compaction of the cells; and the TE on a scale of A to C, according to the number, size, and contiguous arrangement of the TE cells.

Vitrification and Warming of Blastocysts

Vitrification and warming of blastocysts was performed using an unmodified Cryotop method, as described by the manufacturer (Kitazato, BioPharma) and Zhu et al. (12). The method uses high-concentration cryoprotectants with ultrarapid vitrification and warming rates. The equilibration, vitrification, thawing, diluent, and washing solutions required for the procedures are provided in the commercially available Cryotop Safety Kits. Briefly, blastocysts were taken through

an equilibration step and a vitrification step at room temperature before being placed on a Cryotop strip and plunged into liquid nitrogen. No more than two blastocysts were placed on a strip. The Cryotop strip was placed in a cap under liquid nitrogen and stored in a cryotank. For warming, a capped Cryotop container was removed from the cryotank and the strip was removed from the cap under liquid nitrogen. The strip was taken from the liquid nitrogen and plunged into warming solution, and the recovered blastocysts were washed twice. The number of Cryotop containers removed per patient was determined by the intention to have two viable blastocysts for transfer. The blastocyst survival rate in the cycles included in the study was 96.1% (1,022/1,063), with all viable blastocysts not transferred revitrified. The warmed blastocysts were assessed for survival and had their morphologies rescored after a 2-hour equilibration period; these postequilibration blastocyst scores were used in the FET cycle selection.

FET Programming, Blastocyst Transfer, and Luteal Phase Support

All FET cycles were programmed HT cycles, in which the endometrium was prepared and synchronized for transfer, using estrogen (Estrofem, Novo Nordisk) and P (Crinone, Merck Serono) supplements. The cycles were programmed with oral contraceptive pill therapy. Endometrial preparation was performed with a step-up regimen of estrogen supplementation (2 to 8 mg/d). A transvaginal ultrasound scan was performed on day 14 to measure endometrial thickness, and blood was taken to measure the P concentration (26). If the endometrium was >7 mm and there was no evidence of luteinization (<2 ng/mL P), estrogen supplementation (6 mg/d) was continued, vaginal micronized P (Crinone, 8% twice daily [BD]) supplementation was started on day 15, and the transfer of vitrified-warmed blastocysts was performed on the sixth day of P.

All blastocysts selected for transfer were placed in culture medium droplets covered with light mineral oil and placed in incubation until transfer. Blastocyst transfer procedures were performed using a Hamilton syringe (50 μL, Hamilton Company) attached to an embryo replacement catheter (Wallace, Smiths Medical International) and transabdominal ultrasound guidance. Luteal phase support in both fresh and vitrified-warmed cycles consisted of an estrogen (Estrofem, 6 mg/d) and P (Crinone, 8% BD) supplement regimen, which continued to at least 9 weeks of gestation in pregnant cycles (27).

Outcome Measures

The outcomes measured were specific subtypes of pregnancy loss (miscarriage, perinatal mortality) and delivery (preterm delivery, live-birth delivery, live-birth weights, and live-birth gestation), analyzed according to the following definitions. A chemical pregnancy loss was defined as a serial increase in βhCG from a day 14 βhCG level of >30 IU followed by a decline in βhCG levels to below 5 IU/L before 6 weeks of gestation. A clinical pregnancy was a pregnancy with a fetal heart observed on ultrasound scan after 6–8 weeks of gestation. An ectopic pregnancy was a pregnancy with an extrauterine gestational sac observed on ultrasound scan or by laparoscopy. A monochorionic pregnancy was defined as a pregnancy with two fetal hearts in a single fetal sac observed on ultrasound scan. A miscarriage was the spontaneous loss of a clinical pregnancy before the 20th week of gestation. A stillbirth was the delivery of a deceased infant after 20 weeks of gestation. A very preterm delivery was the delivery of an infant before 32 weeks of gestation, and a preterm delivery was a delivery before 37 weeks of gestation. A live birth was the delivery of a live infant after 20 weeks of gestation who survived for at least 7 days. The live delivery of a singleton and a twin pregnancy was counted as one live birth. LBW was a live infant delivered with a weight of less than 2,500 g, and very low birth weight (vLBW) was a live infant delivered with a weight of less than 1,500 g. All obstetric patient care was provided by obstetricians who operated independently of Antalya IVF and were given information regarding the origin of the pregnancy. The outcome rates presented in the study were calculated where appropriate as per transfer or per pregnancy, and the implantation rates were calculated as the ratio of the number of implantations over the number of blastocysts transferred. Gestational age was the number of days (in weeks) between ET and end of pregnancy plus 20 days.

Statistical Analysis

MedCalc version 13.0.6 was used for statistical analysis and to obtain the confidence intervals and risk ratios. Descriptive statistics were presented as the mean and SD for continuous data and as percentages for the categorical data. The independent samples *t*-test was used to compare the means, and the χ^2 or Fisher's exact test was used for to determine statistical significance between percentages. $P<.05$ was considered statistically significant.

RESULTS

Demographic Data

Initially, 1,242 treatment cycles were included in the study, 821 fresh ET and the 421 FET. The pregnancy rates obtained in the two groups were 64.6% (530/821) and 81.7% (344/421), respectively. In the follow-up analysis, 76 (6.1%) of the treatment cycles had to be removed from the analysis because of incomplete data. In this follow-up we were therefore able to analyze the peri-implantation and perinatal outcomes in 784 fresh ET and 382 FET. In the revised groups, the fresh ET pregnancy rate (63.6%, 499/784) was similarly significantly lower (relative risk [RR] = 0.77; $P<.0001$) than the FET pregnancy rate (82.7%, 316/382). The patient demographic data of the included treatment cycles were mostly nonsignificantly different between fresh ET and FET. The variables known to be predictive of pregnancy, that is, patient age (30.2 ± 5.48 vs. 30.5 ± 5.23 years; $P=.373$), infertility duration (4.7 ± 3.67 vs. 4.8 ± 3.80 years; $P=.666$), body mass index (25.6 ± 7.53 vs. 25.5 ± 4.74 kg/m^2; $P=.739$), and obstetric parity (0.170 ± 0.496 vs. 0.175 ± 0.776; $P=.6272$) illustrate this relative parity between the two

transfer groups. The total antral follicle counts (AFC) in the fresh ET cycles were, however, significantly lower (21.4 ± 13.94 vs. 31.0 ± 18.12; $P<.0001$) than in the FET cycles. This outcome was not unexpected as 90% of FET cycles were from patients who had superfluous day 5 blastocysts and 10% were from patients who had freeze-all cycles. The etiological classifications for treatment were also nonsignificantly different, that is, male factor infertility (37.5% vs. 37.7%; $P=.949$), unexplained infertility (33.9% vs. 34.2%; $P=.895$), anovulation (14.0% vs. 15.5%; $P=.535$), tubal infertility (10.2% vs. 8.0%; $P=.288$), decreased ovarian reserve (0.52% vs. 0.53%; $P>.05$), endometriosis (0.52% vs. 0.53%; $P>.05$), and other (3.4% vs. 3.5%; $P>.05$). OHSS, a risk factor for adverse perinatal outcome in fresh ET, occurred in only nine of the fresh ET cycles (1.15%).

Peri-implantation

The mean endometrial thicknesses (9.58 vs. 9.45; $P=.488$), measured on or close to the day of hCG trigger in the fresh ET group and on day 14 of estrogen supplementation in the FET group, were nonsignificantly different. The fresh blastocyst dual embryo transfers (DETs) were performed on the fifth day after the day of the OPU, and the vitrified-warmed blastocyst DETs were performed on the sixth day of P supplementation in HT cycles. The blastocyst implantation rate in the fresh ET group was significantly lower than the implantation rate in the FET group (40.6% vs. 51.5%; RR = 0.79; $P<.0001$), and this reduced implantation outcome contributed to the significantly lower clinical pregnancy rate (54.7% vs. 70.7%; RR = 0.77; $P<.0001$) in the fresh ET group. In addition to the lower blastocyst implantation outcome observed in the fresh ET group, a reduced blood βhCG level (day 14) per blastocyst implantation was also observed (148.2 vs. 176.2 IU/L; $P<.0001$). This reduced βhCG production per implantation in fresh ET was corroborated when cycles were delineated between singleton (154.7 vs. 184.0 IU/L; $P=.005$) and twin (141.2 vs. 167.2 IU/L; $P=.002$) implantations (Table 1). The day 14 βhCG concentrations were 26–29 IU lower for fresh ET implantations. In contrast, the chemical pregnancy loss rates (8.02% vs. 8.86%; $P=.698$) and the monochorionic twinning rates (2.61% vs. 2.53%; $P>.05$) were comparable between the two groups. Although the rate was not signficantly higher, the ectopic pregnancy rate risk (RR = 5.70; 1.8% vs. 0.32%; $P=.098$) in the fresh ET group was clinically significant.

Perinatal

To analyze and compare the perinatal outcomes between fresh ET and FET, we tabulated fresh ET and FET cycles according to fetal heart number, that is, singleton intratuterine and twin intrauterine pregnancies. The twin pregnancy rates calculated per transfer were not significantly (RR = 0.83; $P=.061$) different between fresh ET (26.5%) and FET (32.0%). The perinatal outcomes analyzed are presented in Tables 1–3. The majority of the perinatal outcomes, in both the singleton and the twin analyses, were nonsignificantly different between fresh ET and FET. This included the perinatal outcomes of miscarriage (6–14 weeks and 15–20 weeks), stillbirth, very preterm delivery, preterm delivery, live births per pregnancy, and the mean gestational age of live births. The live birth rates per pregnancy for singletons were 83.4% and 85.7% ($P=.647$), and for twins, 87.1% and 83.1% ($P=.332$). This was in contrast to the live-birth rates per transfer, where the live-birth rate for fresh ET was significantly lower than for FET (44.3% vs. 56.8%; RR = 0.78; $P=.0001$). The male:female ratios of live births, calculated from the total number of live infants delivered from fresh ET and FET, were 1:0.95 (226:215) and 1:0.93 (138:128), respectively.

Birth Weight

In this study the only perinatal outcomes to show significant differences between fresh ET and FET were the live-birth weight outcomes (Table 3). Overall, the live-birth weights were significantly lower for fresh ET, 3,113.8 versus 3,223.5 g for singletons ($P=.044$) and 2,230.0 versus 2,354.0 g for twins ($P=.038$; Table 1). The weight differences when analyzed according to gestational age show that the difference increases with increasing gestational age (Table 3). In the singleton analysis, a weight difference of 152 g, and in the twin analysis, a weight difference of 166.7 g was obtained at 37–40 gestation weeks, while the difference at 33–36 weeks was −8.5 and 86 g, respectively. In the twin comparison a difference of 263.9 g was observed in the <32 week category owing to significantly more LBW (10.7% vs. 3.95%; $P=.016$) and vLBW (62.2% vs. 54.6%; $P\leq.0001$) infants being delivered in the fresh ET group.

Subanalyses

Three subanalyses were performed to investigate specific subgroups from the fresh ET and FET groups, the outcomes of which are presented in Supplemental Tables 1–3. Grouping the live-birth weights of infants according to sex, it was seen that the live-birth weights of male infants were significantly higher than the live-birth weights of female infants from fresh ET, for both singletons (3,179.4 vs 3,036.5 g; $P=.017$) and twins (2,330.0 vs. 2,131.5 g; $P=.003$; Supplemental Table 1). In contrast, the live-birth weights of male and female infants from FET were nonsignificantly different: 3,220.4 versus 3,226.0 g for singletons ($P=.955$) and 2,359.8 versus 2,347.0 g for twins ($P=.873$). The live-birth weights of both male and female infants were lower from fresh ET compared with their counterparts from FET. The differences in birth weights for female infants (189.5–215.5 g) were significant for both singletons ($P=.021$) and twins ($P=.004$), while the differences (29.8–1.0 g) for male infants were nonsignificant. These differences in male and female live delivery weight outcomes were also reflected in the day 14 βhCG blood concentrations for male and female implantations. The mean βhCG concentration for male infants was 167.8 IU/L, and for female infants it was 150.8 IU/L from fresh ET, while the means from FET were 185.1 and 184.2 IU/L, respectively.

TABLE 1

Reproductive outcomes of singleton and twin pregnancies from fresh ET and FET.

Reproductive outcomes	Fresh ET, n = 205[a] singleton pregnancies	FET, n = 133[a] singleton pregnancies	RR	*P* value (95% CI)	Fresh ET, n = 202[a] twin pregnancies	FET, n = 124[a] twin pregnancies	RR	*P* value (95% CI)
βhCG IU/fetal heart, mean[b] (SD)	154.7 (82.79)	184.0 (108.27)		.005 (8.79–49.80)	141.2 (69.22)	167.2 (76.91)		.002 (9.79–42.21)
Miscarriage rate (6–14 wk), % (n)	10.2 (21/205)	9.77 (13/133)	1.05	>.05 (0.54–2.02)	3.47 (7/202)	8.87 (11/124)	0.39	.474 (0.156–0.981)
Miscarriage rate (15–20 wk), % (n)	3.90 (8/205)	3.01 (4/133)	1.30	.770 (0.40–4.22)	4.46 (9/202)	4.03 (5/124)	1.11	.151 (0.379–3.22)
Total miscarriage rate, % (n)	14.1 (29/205)	12.8 (17/133)	1.11	.749 (0.63–1.93)	7.92 (16/202)	12.9 (16/124)	0.61	.446 (0.319–1.18)
Stillbirth rate (>20 wk), % (n)[c]	2.44 (5/205)	1.50 (2/133)	1.62	.708 (0.32–8.23)	8.4 (17/202)	5.65 (7/124)	2.95	.391 (1.27–6.84)
Live birth, % (n)	83.4 (171/205)	85.7 (114/133)	0.97	.647 (0.89–1.07)	87.1 (176/202)	83.1 (103/124)	1.05	.332 (0.953–1.15)
Twin delivery, % (n)					66.83 (135/202)	61.3 (76/124)	1.09	.340 (0.920–1.29)
Delivery	176	116			186	108		
Gestational age (wk), mean (SD)[d]	37.93 (1.506)	37.98 (1.347)		.756 (−0.27 to 0.37)	35.57 (2.992)	35.85 (2.441)		.381 (−0.347 to 0.907
Birth weight (g), mean (SD)[d]	3,113.8 (453.27)	3,223.2 (530.50)		.044 (3.16–215.6)	2,230.0 (546.26)	2,354.0 (481.52)		.038 (6.90–242.1)
Male:female ratio	1:0.86	1:1.04			1:1.01	1:0.85		

[a] Excluding cycles with terminations/reductions, ectopic pregnancies, and monochorionic multiple pregnancies.
[b] The mean βhCG concentration calculated from the day 14 (only) βhCG concentrations per fetal heart implantation.
[c] The loss of at least one fetus after 20 wk of gestation.
[d] Live births.

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.

Selecting the transfers in which at least one blastocyst transferred had grade 5 expansion, the comparative analysis was repeated between fresh ET and FET (Supplemental Table 2). The peri-implantation and the perinatal outcomes from fresh ET and FET in this analysis mirrored those obtained in the overall analysis, that is, pregnancy (69.6% vs. 87.5%; $P<.0001$), clinical pregnancy rate (59.9% vs. 79.4%; $P\leq.0001$), live birth per transfer (49.4% vs. 64.0%; $P=.002$), singleton live-birth weights (3,143.4 g vs. 3,271.7 g; $P=.537$), and twin live-birth weights (2,336.5 vs. 2,464.4 g; $P=.017$) were all lower in fresh ET. In this select comparison, a 0% (0/140) ectopic pregnancy rate was obtained from FET, while the ectopic pregnancy rate for fresh ET was 1.55% (5/323).

The AFC was found to be significantly lower in the fresh ET group (21.4 vs. 31.0; $P<.0001$), and this was also reflected in a significantly lower oocyte recovery rate at OPU in the fresh ET cycles (16.5 ± 8.54 vs. 22.8 ± 13.09; $P<.001$). This subanalysis was, therefore, performed to assess whether greater AFC parity would alter the peri-implantation outcomes. Selecting treatment cycles where patients had AFCs ranging between 12 and 30, we repeated the comparative analysis between fresh ET and FET and presented them in Supplemental Table 3. Although the AFC was still significantly different (19.5 vs. 20.7; $P=.024$), the magnitude of difference was smaller than in the overall study. The peri-implantation outcomes observed for fresh ET and FET, however, mirrored those obtained in the overall analysis, that is, pregnancy (59.2% vs. 81.8%; $P<.0001$), clinical pregnancy (51.3% vs. 73.6%, $P<.0001$), implantation rate (38.4% vs. 55.0%; $P=.0001$), and βhCG IU/fetal heart (210.8 vs. 242.1 IU; $P=.032$) were also significantly lower in fresh ET.

DISCUSSION

In the present study we examined the peri-implantation and perinatal outcomes as well as the birth weights of infants conceived after either fresh or vitrifed-warmed transfers of similar good-quality (grade ≥3) blastocysts. The choice of using only day 5 blastocysts and at least one blastocyst with an expansion grade of ≥3 was a deliberate attempt to reduce the possible effect that embryo-endometrium asynchrony may have on the pregnancy outcomes of fresh ET. The main outcomes of the study showed that FET resulted in significantly higher implantation rates (51.5% vs. 40.6%), live-birth rates (56.8% vs. 44.3%), live-birth weights for both singletons (3,223.2 vs. 3,113.8 g) and twins (2,350.0 vs. 2,230.0 g), and a lower ectopic pregnancy rate (0.32% vs. 1.80%). The live-birth weight differences were greater for female infants than for male infants, that is, a weight difference of 190 g for female singletons compared with a weight difference of 41 g for male singletons. Importantly, the comparative analysis also showed that the perinatal outcomes of miscarriage, premature delivery, and perinatal morbidity in both singleton and twin pregnancies were comparable between fresh ET and FET.

Our analysis suggests that the peri-implantation conditions may play a significant role in the differences observed

TABLE 2

Delivery outcomes from singleton and twin pregnancies of fresh ET and FET.

Delivery outcomes	Fresh ET, n = 176[a] singleton pregnancies	FET, n = 116[a] singleton pregnancies	RR	*P* value (95% CI)	Fresh ET, n = 186[b] twin pregnancies	FET, n = 108[b] twin pregnancies	RR	*P* value (95% CI)
Very preterm delivery (<32 wk), % (n)	3.41 (6/176)	2.6 (3/116)	1.32	>.05 (0.336–5.17)	15.1 (28/186)	14.8 (16/108)	1.02	.867 (0.577–1.79)
Preterm delivery (<37 wk), % (n)	19.9 (35/176)	18.1 (21/116)	1.10	.763 (0.675–1.79)	65.1 (121/186)	65.7 (71/108)	0.99	>.05 (0.833–1.18)
Term delivery (≥37 wk), % (n)	80.1 (141/176)	81.9 (95/116)	0.98	.763 (0.874–1.10)	34.9 (65/186)	34.3 (37/108)	1.02	>.05 (0.736–1.41)

Note: Including cycles with one fetal heart[a] and with two fetal hearts[b] that had deliveries after 22 wk of gestation, excluding cycles with terminations/reductions, ectopic pregnancies, and monochorionic multiple pregnancies.

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.

between fresh ET and FET. The blastocyst implantation rate (51.5% vs. 40.6%; $P<.0001$) and the βhCG concentrations (176.2 vs. 148.2; $P=.0001$) per fetal heart implantation were significantly higher in FET than in fresh ET. The significantly higher mean day 14 βhCG concentrations per implantation observed in the FET cycles were suggestive of a difference in embryonic regulation of βhCG expression and secretion (28, 29), and as the blastocysts transferred were all of good and similar quality, the increased hCG secretion may be more a function of greater endometrial competence (1, 29, 30). The findings of our study, therefore, support the presupposition that a more physiological early luteal phase will increase the chances of implantation and improve early placentation and embryogenesis and as a consequence may differentially impact fetal growth. The latter was affirmed by the higher live-birth weights observed in FET pregnancies, both in singleton (Δ109 grams) and twin (Δ124 grams) pregnancies.

The precise "how and which" aspects of endometrial receptivity and embryo implantation ART most significantly impacts still remain uncertain. Moreover, how this impact is moderated by parental characteristics is also largely unknown (3, 7, 21, 31). In fresh ET, the supraphysiological hormonal conditions produced by COS certainly have the potential to adversely affect many aspects of early conception, that is, perifollicular, peri-ovulatory, and peri-implantation function and development (2, 3, 5, 6, 16–18). In studies comparing cohorts of patients with different COS response levels, it was found that increased levels of estrogen and P were associated with increased risks of pregnancy complications (3, 6, 17). The observed pregnancy complications were mostly related to abnormal placentation, that is, placenta previa, pregnancy-induced hypertension, preterm premature rupture of membranes, and fetal growth restriction (6, 32). On the other hand, the effect of cryopreservation technology on embryo survival, embryogenesis, and infant health has also been a concern. Recent studies have shown that improvements in cryopreservation technology have resulted in improved embryo survival and implantation, without significantly increasing any adverse short-term health outcomes for infants born (9, 11, 13, 33).

After implantation and the confirmation of a fetal heart, the perinatal outcomes of pregnancies from fresh ET in our study were similar to those from FET. The rates of miscarriage, stillbirth, preterm delivery, live birth, and mean gestational age of live births were all not significantly different between the two transfer groups—both in singleton and twin pregnancies. The outcomes of our study can best be compared with the population-based comparative studies by Li et al. (33) (Australia and New Zealand; fresh versus vitrification blastocyst transfers), and Ishihara et al. (8) (Japan; fresh versus thawed blastocyst transfers). Even though there may be important differences between our study and theirs, that is, a lower mean patient age (≈30 years vs. 34–36 years) and a greater difference in clinical pregnancy favoring FET (16.0% vs. 3.2%–8.5%), all reported similar comparative outcomes. Differences in live birth, miscarriage, infant mortality, and gestational age of live births from singleton pregnancies were mostly not significant, while differences in birth

TABLE 3

Live delivery weights of infants from singleton and twin pregnancies of fresh ET and FET.

Delivery weight	Fresh ET, n = 171[a] singleton pregnancies	FET, n = 114[a] singleton pregnancies	RR	*P* value (95% CI)	Fresh ET, n = 270[b] twin pregnancies	FET, n = 152[b] twin pregnancies	RR	*P* value (95% CI)
<32 wk	1,200[b]	2,300[b]			1,361.4 (334.92)	1,625.3 (345.0)		<.0001 (196.4–331.4)
33–36 wk	2,860.3 (384.30)	2,851.8 (352.81)		.850 (−97.05 to 80.05)	2,177.5 (408.33)	2,263.5 (387.55)		.0350 (6.08–165.9)
37–40 wk	3,180.0 (419.38)	3,332.3 (482.30)		.005 (46.25–258.3)	2,518.1 (383.94)	2,684.8 (572.47)		.0004 (74.87–258.5)
vLBW (<1,500 g), % (n)	0.58 (1/171)	0.0 (0/114)	2.01	>.05 (0.082–48.81)	10.7 (29/270)	3.95 (6/152)	4.59	.016 (1.96–10.75)
LBW (<2,500 g), % (n)	7.01 (12/171)	7.02 (8/114)	1.00	>0.05 (0.422–2.37)	62.2 (168/270)	54.6 (83/152)	1.81	<.001 (1.56–2.09)

Note: In the singleton groups, one macrosomic infant was delivered in each of the groups. In the twin groups, no macrosomic infants were delivered.

[a] Including cycles with the live delivery of a singleton and a twin, excluding cycles with terminations/reductions, ectopic pregnancies, and monochorionic multiple pregnancies.

[b] Only one live delivery in each of the two groups at <32 wk of gestation.

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.

weight–related outcomes were significant. These and other population-based studies have been published wherein the perinatal and obstetric outcomes from national registries (births and deaths) were used to compare fresh ET and FET pregnancy outcomes. In contrast to our study, heterogeneous procedures and technologies were often used in the conceptions analyzed, that is, ETs performed at different developmental stages, different cryopreservation technologies and methods, and GnRH agonist or GnRH antagonist cotreatment. Nonetheless, the outcomes reported have been consistent, with FET pregnancies reported to have reduced preterm delivery (8, 21, 22), very preterm delivery (22), LBW infant (8, 21–23), small for gestational age infant (8, 22) risks, increased high birth weight (21), large for gestational age infant (8, 22, 23), macrosomia (22–34), perinatal mortality (22, 23), and post-term delivery (22) risks relative to fresh ET pregnancies. Five of the outcomes reported were primarily weight related, which was consistent with the observations in our single-center study. These differences in risks were observed to persist even after adjusting for prognostic confounding variables, that is, duration of infertility, maternal age, parity, year of birth, infant sex, and birth order (4, 21, 22). In the subanalyses (AFC and blastocyst grade) performed in the present study, the peri-implantation outcome rates may have increased within the groups, but the differences in the peri-implantation and perinatal outcomes between the groups remained relatively constant (Supplemental Tables 2 and 3).

The aspect of clinical importance observed was the possible association between placentation and fetal growth-determining factors—and the observation that these aspects were enhanced in FET. In animal studies this link between impaired placentation, induced by superovulation conditions, and fetal growth modifications has been confirmed (3). Fetal growth in superovulation pregnancies has been observed to be restricted in early to mid term, while in the end term a significant increase in placental size occurs, resulting in accelerated fetal growth. These changes in fetal development were suggested to be a compensatory reaction to poor placentation (22). In the present study the difference in birth weight reached greater significance after 37 weeks of gestation, with a difference of 152 g for singleton and 167 g for twin pregnancies. This greater growth compensation in FET may be the consequence of the use of either cryopreservation technology or HT or both. In addition, in the subanalysis using live-birth weight outcomes, arranged according to infant gender (Supplemental Table 1), we found sex to be a significant growth-determining factor. While male infant live-birth weights, in pregnancies from both fresh ET and FET pregnancies, were greater than for female infants, the actual difference between male and female birth weights in FET was marginal (Δ6–12 g) in comparison with the difference observed in fresh ET (Δ143–199 g). The live birth weights of female infants (190–215 g) also showed a greater increase in weight between fresh ET and FET compared with male infants (30–41 g).

Another important difference between fresh ET and FET pregnancy outcomes is the lower ectopic pregnancy risk in FET. In a recent study designed specifically to compare ectopic pregnancy outcomes between fresh ET and FET, a

rate of 4.62% was obtained in fresh ET versus a 1.05% rate in FET (35). The ectopic pregnancy rates we obtained were, however, 1.80% in fresh ET and 0.32% in FET. These lower ectopic pregnancy rates may be a result of the fact that only blastocysts were transferred in our study, a phenomenon corroborated by the ectopic pregnancy outcomes reported in the studies by Li et al. (33) and Ishihara et al. (8). Furthermore, we observed that blastocyst quality or blastocyst development stage may further moderate this rate, as no (0%) ectopic pregnancies were obtained in the 160 FETs performed where at least one of the two blastocysts transferred had an expansion grade of 5.

A limitation of the study is that it is a single-center perinatal study, and by limiting the time frame of the study to ensure greater homogeneity, not all variables may be adequately powered to show statistical significance. A case in point is the statistical analysis of ectopic pregnancy in the present study. However, we still regard the outcomes presented in our study to be of clinical value. The main reasons being that the outcomes were from cycles with controlled patient fertility variables (i.e., age, years of infertility, body mass index, parity, and etiology) and pregnancy variables (i.e., the number and quality of blastocysts transferred) and most importantly with limited protocol and procedure heterogeneity (i.e., COS, in vitro embryo culture, blastocyst transfer, blastocyst cryopreservation, and HT protocol). The two groups were essentially differentially affected by only two confounding variables, COS and blastocyst cryopreservation. While there is probably sufficient evidence confirming the adverse affects of COS on the early luteal phase and consequently as seen in our study on embryogenesis, there is now evidence that in vitro manipulations and cryopreservation technology may also affect embryo development processes and subsequently intrauterine fetal growth (7) and, therefore, the outcomes observed in our study.

In conclusion, this study showed that blastocyst FET results in better pregnancy, implantation, and possibly better fetal growth outcomes as compared with fresh blastocyst ET, with suggestions of superior endometrial receptivity and placentation in FET. Poor perinatal outcomes are known to be associated not only with increased neonatal morbidity but also with long-term health outcomes, including an increased risk of metabolic syndrome–related diseases. Further studies are therefore vital to investigate the sources of and the mechanisms involved in the health-related epigenetic modifications that occur through ART, as the main goal of ART is the delivery of healthy infants.

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SUPPLEMENTAL TABLE 1

Male and female live delivery weight comparisons for fresh ET and FET.

Live birth weight	Fresh ET male	Fresh ET female	Fresh ET male vs. female, *P* value (95% CI)	FET male	FET female	FET male vs. female, *P* value (95% CI)	Fresh ET vs. FET, male vs. male and female vs. female, *P* value (95% CI)
Live-birth weights (singleton pregnancies), mean (SD)	n = 92, 3,179.4 (470.5)	n = 79, 3,036.5 (422.0)	.017 (−260.5 to 25.28)	n = 56, 3,220.4 (536.0)	n = 58, 3,226.0 (529.8)	.955 (−192.2 to 203.4)	.627 (−125.2 to 207.2); .021 (28.60–350.4)
Live birth weights (twin pregnancies), mean (STD)	n = 134, 2,330.0 (560.0)	n = 136, 2,131.5 (515.4)	.003 (327.4–69.56)	n = 82, 2,359.8 (506.8)	n = 70, 2,347.0 (452.8)	.873 (−167.8 to 142.6)	.710 (−127.9 to 187.5); .004 (71.90–359.1)

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.

SUPPLEMENTAL TABLE 2

Reproductive outcomes of singleton and twin pregnancies from fresh ET and FET, where at least one blastocyst had grade 5 expansion.

Reproductive outcomes	Fresh ET, n = 464 transfers	FET, n = 160 transfers	RR	*P* Value (95% CI)
Pregnancy, % (n)	69.6 (323/464)	87.5 (140/160)	0.80	< .0001 (0.732–0.865)
Clinical pregnancy, % (n)	59.9 (278/464)	79.4 (127/160)	0.74	< .0001 (0.677–0.84)
Chemical pregnancy loss, % (n)	8.05 (26/323)	5.0 (7/140)	1.63	.179 (0.816–4.15)
Live birth per transfer, % (n) [a]	49.4 (223/451)	64.0 (96/150)	0.77	.002 (0.664–0.899)
Implantation rate, % (n) [b]	45.3 (413/912)	59.4 (183/308)	0.76	< .0001 (0.678–0.857)
βhCG IU/fetal heart, mean (SD)[a,c]	161.2 (82.0)	189.4 (106.26)		.0006 (12.20–44.19)
Singleton pregnancy				
βhCG IU/fetal heart, mean (SD)	163.6 (90.41)	190.3 (95.83)		.0016 (10.17–43.23)
Live birth per pregnancy, % (n)	80.2 (97/121)	82.6 (43/52)	0.69	.834 (0.832–1.129)
Birth weight, mean (SD) [d]	3,143.4 (465.35)	3,271.7 (588.06)		.537 (−61.65 to 118.25)
Twin pregnancy				
βhCG IU/fetal heart, mean (SD)	147.0 (60.67)	182.1 (86.46)		.466 (−59.46 to 129.7)
Live birth per pregnancy, % (n)	84.0 (121/144)	83.1 (54/65)	0.86	.842 (0.887–1.153)
Birth weight, mean (SD) [d]	2336.5 (594.18)	2464.4 (539.19)		.017 (23.37–232.4)

Note: The monochorionic pregnancy rate was 4.7% in the vitrified-warmed transfer group and 2.48% in the fresh transfer group.
[a] Excluding cycles with terminations/reductions and monochorionic multiple pregnancies.
[b] Excluding cycles monochorionic multiple pregnancies.
[c] The mean βhCG concentration calculated from the day 14 (only) βhCG concentrations per fetal heart implantation.
[d] Live births.

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.

SUPPLEMENTAL TABLE 3

Reproductive outcomes in transfer cycles with AFC ranging between 12 and 30.

Reproductive outcomes	Fresh ET, n = 314 transfers	FET, n = 121 transfers	RR	95% CI
AFC, mean (SD)	19.46 (5.217)	20.74 (5.450)		.024 (0.169–2.391)
Pregnancy, % (n)	59.2 (186/314)	81.8 (99/121)	0.72	<.0001 (0.639–0.820)
Clinical pregnancy, % (n)	51.3 (161/314)	73.6 (89/121)	0.70	<.0001 (0.600–0.811)
Chemical pregnancy loss, % (n)	6.99 (13/186)	5.05 (5/99)	1.38	.616 (0.508–3.77)
Implantation rate, % (n) [a]	38.4 (241/628)	55.0 (133/242)	0.72	.0001 (0.621–0.838)
βhCG IU/fetal heart, mean (SD) [b,c]	210.8 (132.7)	242.1 (142.9)		.032 (2.78–59.82)

[a] Excluding cycles with monochorionic multiple pregnancies.
[b] Excluding cycles ectopic pregnancies and monochorionic multiple pregnancies.
[c] The mean βhCG concentration calculated from the day 14 (only) βhCG concentrations per fetal heart implantation.

Ozgur. Perinatal outcomes from fresh ET and FET. Fertil Steril 2015.